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Measurement and pharmacokinetic study of tetramethylpyrazine in rat blood and its regional brain tissue by high-performance liquid chromatography

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Abstract

We used a rapid, sensitive and reliable high-performance liquid chromatographic method for the determination of tetramethylpyrazine in rat brain tissue and plasma. The lower limit of quantification in plasma and brain tissue was 0.1 μg/ml and 0.1 μg/g, respectively, and only a small amount of plasma (100 μl) or brain tissue (100 μg) was required for analysis. The decline in the concentration of tetramethylpyrazine in plasma was generally two-exponential at a dose of 2, 5, or 10 mg/kg administered intravenously. Concentrations of tetramethylpyrazine in various regions of the brain (cerebral cortex, brainstem, striatum, hippocampus, cerebellum and midbrain) were not significantly different at 15 min following drug administration (10 mg/kg, i.v.). In additional analysis, mean concentration of the tetramethylpyrazine in rat plasma was approximately five-times greater than the drug in brain tissue. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Tetramethylpyrazine, one of the active principles isolated from the traditional medicinal herbs *Ligusticum wallichii* Franch. or *L. chuanxiong* Hort, has been widely used in China for the treatment of cardiovascular and cerebrovascular disease [1] and is known to increase the cerebral blood flow and ischemic attack [2]. It has been reported recently that tetramethylpyrazine may ameliorate learning deficit induced by permanent occlusion of the bilateral common carotid arteries in rats [3] and its effectiveness as a cognitive enhancer has also been demonstrated [4]. These findings strongly suggest that tetramethylpyrazine has an effect on the central nervous system, and as a first step, we are therefore concerned here with measuring drug concentration in rat brain tissue.

Several studies have reported for the determination of tetramethylpyrazine by ultraviolet spectrophotometry [5], high-performance liquid chromatography (HPLC) [6], and gas chromatography (GC) and gas chromatography–mass spectrometry (MS) [7]. However, not all of the above methods measured the tetramethylpyrazine in rat plasma and brain tissue. Because of its therapeutic potential in peripheral

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circulation and central brain systems, intensive investigations are warranted, there is no extant method of its quantitation. This paper describes a simple and sensitive liquid chromatography method with ultraviolet (UV) detection to determine the concentration of tetramethylpyrazine in rat plasma and its related pharmacokinetic profile as well as brain tissue distribution.

2. Experimental

2.1. Chemicals

Tetramethylpyrazine and coumarin (internal standard) were purchased from Sigma (St. Louis, MO, USA). Methanol and chromatographic reagents were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations. A standard stock solution of tetramethylpyrazine was prepared by dissolving 1.0 mg of tetramethylpyrazine in 10 ml of methanol. The internal standard (coumarin) solution was prepared in acetonitrile at a concentration of 1 μg/ml.

2.2. Chromatographic system

The HPLC system consisted of a Rheodyne injector, a chromatographic pump (ICI 1100, ICI Instrument, Australia), a UV–Vis detector (ICI 1200), and a data system for chromatogram integration (EZChrom, Scientific Software, San Ramon, CA, USA), all operated at room temperature (24±1°C). Separation was achieved on a Cosmosil 5 C_{18} column (250×4.6 mm, particle size 5 μm). The mobile phase consisted of methanol–water (50:50, v/v, pH 3.0 adjusted by orthophosphoric acid) at a flow-rate of 1.0 ml/min. The mobile phase was filtered before use using a Millipore vacuum filter system equipped with a 0.22-μm filter (Waters). Further degassing was found not necessary immediately after filtration. Tetramethylpyrazine was monitored at a wavelength of 280 nm throughout the experiments.

2.3. Animals

Male Sprague–Dawley rats (300±50 g) were obtained from the Laboratory Animal Center of National Yang-Ming University, Taipei, Taiwan. The rats (specifically pathogen-free) were housed in stainless steel cages and maintained in a controlled environment of a 12-h light–dark cycle (7:00–19:00) with temperature maintained at 24±1°C. Water and standard laboratory food were given ad libitum until 18 h before the experiments, at which time only food was withdrawn.

2.4. Blood sampling and treatment for pharmacokinetics

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and their body temperature maintained at 37°C with a heating pad. The drug was administered in a single bolus through the femoral vein of the rat. Blood samples (0.3 ml) were directly withdrawn via heart puncture of the rat and collected from the same animals at 2.5, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min following tetramethylpyrazine (2, 5 or 10 mg/kg, i.v.) administration. Each blood sample was transferred to a heparinized microfuge tube (1.5 ml) and centrifuged at 3000 *g* for 5 min. The resulting plasma sample (0.1 ml) was votex-mixed with 0.2 ml of internal standard (coumarin, 1 μg/ml) solution. The denatured protein precipitate was separated by centrifugation at 8000 *g* for 5 min. An aliquot (20 μl) of the supernatant was directly injected onto the HPLC apparatus for analysis. Data from these samples were used to construct pharmacokinetic profiles of drug concentration in blood versus time. The same sample handling process was used for the determination of precision [8–10].

To investigate brain distribution of tetramethylpyrazine, rats were killed by decapitation, and various brain parts (cerebral cortex, hippocampus, striatum, cerebellum, brain stem and midbrain) were divided and weighed. The separated brain regions were homogenized with Ringer's solution (1 ml/0.1 g tissue) using a tissue Polytron homogenizer (Kinematica, Switzerland). The homogenate (0.1 ml) of brain tissue was protein denatured by 0.2 ml internal standard solution. After votex-mixing, the

homogenate solution was then centrifuged at 8000 *g* for 5 min. The supernatant (20 μl) was used for HPLC analysis [11].

2.5. Accuracy and precision

The accuracy and precision of the present method were evaluated by analyzing plasma and brain tissue samples spiked with different concentrations of tetramethylpyrazine (0.5, 1, 2, 5 and 10 μg/ml or μg/g). To determine intra-assay variance, samples (six replicates of five different concentrations) were analyzed on the same day. To determine inter-assay variance, samples (five different concentrations) were analyzed on the days 1, 2, 4 and 6 after spiking using a daily calibration curve. The precision and accuracy were expressed as coefficient of variation (CV) and % bias, respectively [8].

2.6. Pharmacokinetic analysis

The plasma concentrations of tetramethylpyrazine versus time profiles were described by compartmental models using the computer program PCNONLIN (version 4.2, SCI Software, Lexington, KY, USA). The following equation applies to a two-compartment open pharmacokinetic model for intravenous bolus:

$$C = A\,e^{-\alpha t} + B\,e^{-\beta t} \quad (1)$$

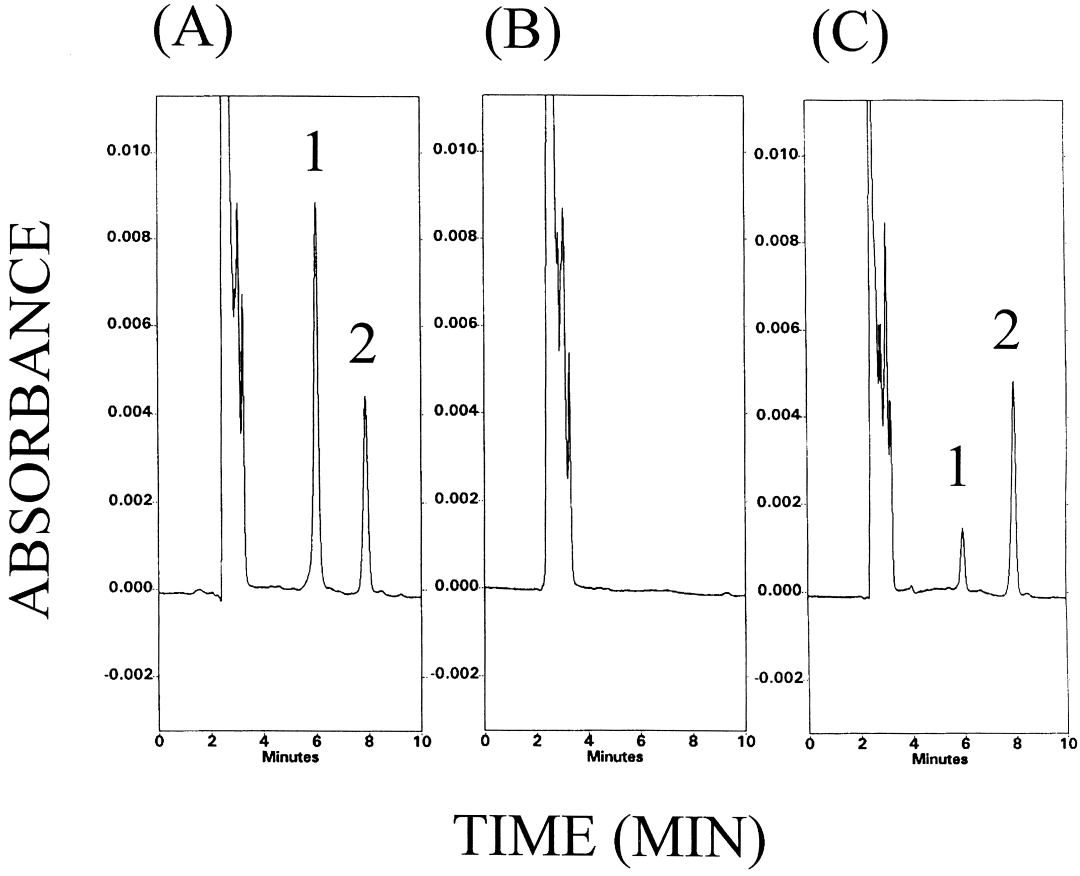

Fig. 1. Chromatograms of rat plasma: (A) plasma spiked with tetramethylpyrazine (5 μg/ml) and coumarin (internal standard; 1 μg/ml). (B) Blank plasma. (C) Plasma sample containing tetramethylpyrazine (0.58 μg/ml) collected from a rat 45 min after tetramethylpyrazine administration (5 mg/kg, i.v.). 1=Tetramethylpyrazine; 2=internal standard (coumarin).

In the above equation, A and B are the concentration (C) intercepts for the fast and slow disposition phases, respectively, and α and β are the disposition rate constants for the fast and slow disposition phases, respectively. Pharmacokinetic parameters such as distribution half-life ($t_{1/2,\alpha}$), elimination half-life ($t_{1/2,\beta}$), area under the concentration curve (AUC), and clearance (CL) were subsequently calculated by the computer program PCNONLIN.

3. Results and discussion

Chromatograms for rat plasma and brain tissue (Figs. 1 and 2, respectively) show that no other discernible peaks were observed within the time frame in which tetramethylpyrazine and internal standard were detected. The retention times of tetramethylpyrazine and internal standard (coumarin) were found to be 5.9 and 7.9 min, respectively (Figs. 1 and 2). The peak area ratios (tetramethylpyrazine to coumarin) of six concentrations of tetramethylpyrazine were linearly related to the concentration of the drug (correlation coefficient, $r^2 = 0.999$) and the equation for the regression line for tetramethylpyrazine was found to be $y = 2.33x - 0.04$. The detection limit for tetramethylpyrazine, at a signal-to-noise ratio of 3:1, was 0.1 μg/ml in rat plasma.

In the method described by Wen et al. [6], tetramethylpyrazine was extracted from serum with

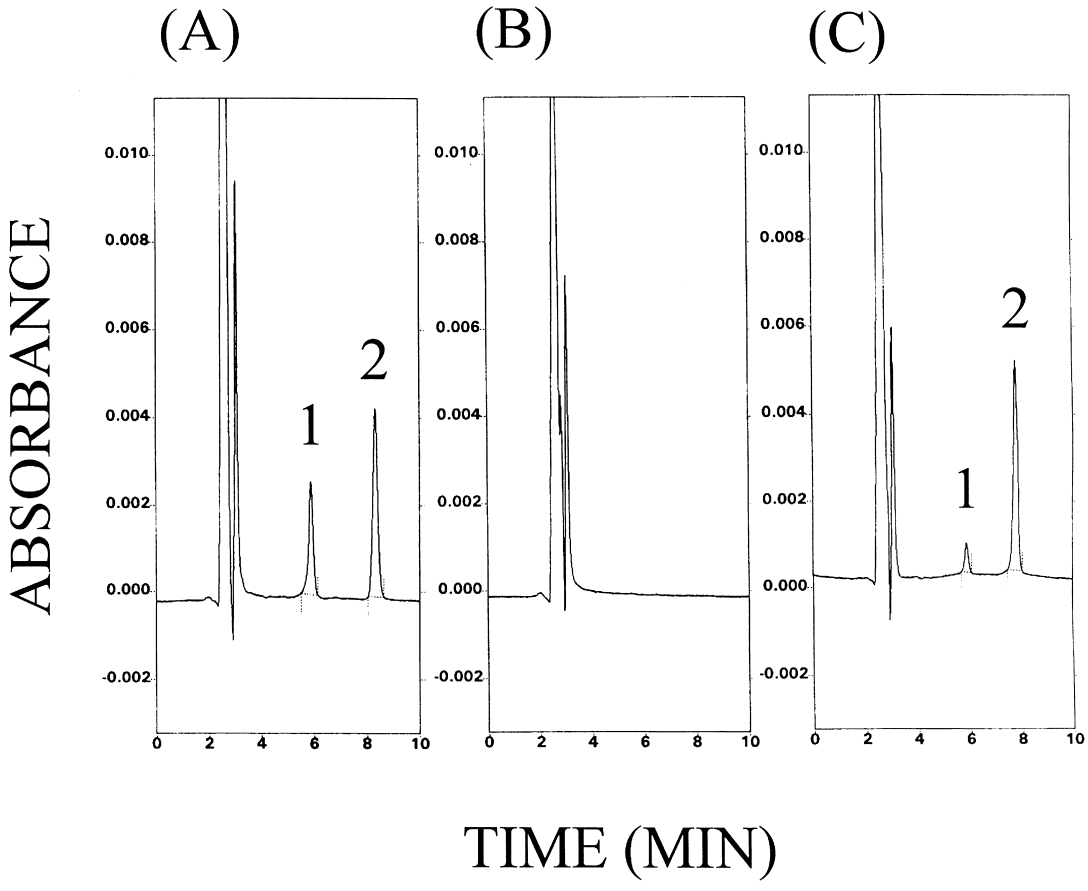

Fig. 2. Chromatograms of rat brain tissue: (A) tetramethylpyrazine (1 μg/g) and internal standard spiked in cerebral cortex homogenate. (B) Blank cerebral cortex homogenate. (C) Homogenized brain sample containing tetramethylpyrazine (0.16 μg/g) collected from a rat cerebral cortex 15 min after tetramethylpyrazine administration (10 mg/kg, i.v.). 1 = Tetramethylpyrazine; 2 = internal standard (coumarin).

chloroform and evaporated to dryness. However, the liquid–liquid extraction of biological sample by organic solvent required more time for the sample matrix treatment. Recently, Sanagi et al. [7] described a supercritical fluid extraction involving GC–MS. The GC–MS technique showed specificity particularly for the measurement of biological sample, but required mass spectrometric equipment.

In our assay, the specific mobile phase for the optimum separation of tetramethylpyrazine from blood and brain tissue samples was methanol–water (50:50, v/v, pH 3.0 adjusted by orthophosphoric acid) in less than 10 min (Figs. 1 and 2). For biological sample treatment, the plasma and brain tissue matrix precipitated by acetonitrile does not interfere with the detection of the tetramethylpyrazine and internal standard peaks, even at this low concentration.

The recoveries of tetramethylpyrazine from rat plasma and rat brain at all the tested concentrations were between 93–99% (Table 1). Both intra-assay and inter-assay variabilities in rat plasma (Table 2) and rat brain (Table 3) were used as measures of the reproducibility of the method. Except at the lowest concentrations in rat plasma, all CVs were less than 5%, and the calculated bias only exceeded 10% in one case (i.e., the inter-assay variability in brain homogenate at the lowest nominal drug concentration).

Drug concentration in plasma was plotted against time after tetramethylpyrazine administration (2, 5 and 10 mg/kg, i.v.; Fig. 3). A statistical nonlinear regression program accessed through the PCNONLIN program was used to compare the pharmacokinetic models (one vs. two compartments) according to Akaike's information criterion (AIC) [12] and the Schwartz criterion (SC) [13]. Minimum AIC and SC values were regarded as the best representation of the plasma concentration–time course data. Because of the apparently two-phasic disposition of tetramethylpyrazine in plasma after intravenous bolus (Fig. 3), a two-compartment open model was proposed, and this was validated by the PCNONLIN

Table 1
Recoveries of tetramethylpyrazine in rat plasma and brain tissue[a]

Concentration	Recovery (%)
Plasma (μg/ml)	
2	93.5±0.2
5	97.1±0.7
10	98.7±0.3
Brain tissue (μg/g)	
1	97.3±0.4
2	97.8±0.6
5	98.2±0.6

[a] Data are expressed as mean±S.E.M. ($n=6$).

Table 2
Precision and accuracy of tetramethylpyrazine in rat plasma ($n=6$)

	Nominal concentration (μg/ml)				
	0.5	1	2	5	10
Intra-assay					
Mean ($n=6$)	0.46	1.0	2.15	5.04	9.97
SD	0.04	0.04	0.03	0.21	0.11
% CV[a]	8.7	4.0	1.4	4.2	1.1
% Bias[b]	−8	0.1	7.5	0.8	−0.3
Inter-assay					
Mean ($n=6$)	0.46	1.05	2.19	4.87	10.01
SD	0.04	0.03	0.05	0.16	0.07
% CV[a]	8.7	2.9	2.3	3.3	0.7
% Bias[b]	−8	5	9.5	−2.6	0.1

[a] Precision (% CV) = 100×(Standard deviation)/(Mean concentration).

[b] Accuracy (% bias) = 100×(Mean concentration − Nominal concentration)/Nominal concentration.

Table 3
Precision and accuracy of tetramethylpyrazine in homogenized rat brain tissue ($n=6$)

	Nominal concentration (μg/g)				
	0.5	1	2	5	10
Intra-assay					
Mean ($n=6$)	0.46	1.04	2.21	4.87	10.01
SD	0.022	0.014	0.069	0.053	0.012
% CV[a]	4.8	1.3	3.12	1.1	0.1
% Bias[b]	−8	4	10	−2.6	0.1
Inter-assay					
Mean ($n=6$)	0.41	1.02	2.22	4.99	9.95
SD	0.02	0.02	0.05	0.09	0.04
% CV[a]	4.9	2	2.2	1.8	0.4
% Bias[b]	−18	2	10	−0.1	−0.5

[a] Precision (% CV) = 100×(Standard deviation)/(Mean concentration).

[b] Accuracy (% bias) = 100×(Mean concentration − Nominal concentration)/Nominal concentration

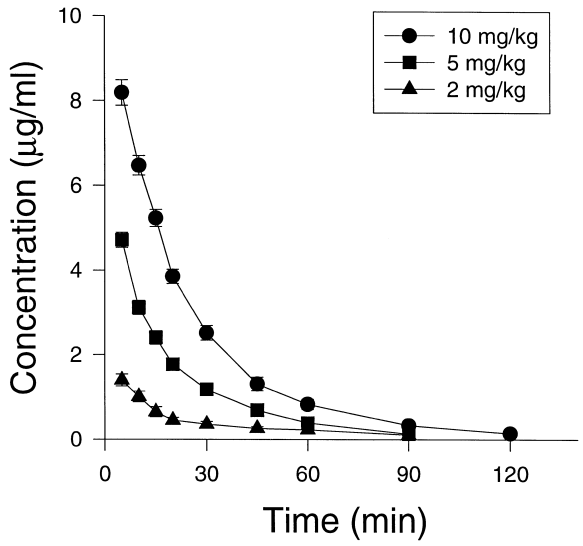

Fig. 3. Plasma concentration–time curve after tetramethylpyrazine administration (2, 5 and 10 mg/kg, i.v.). The corresponding concentrations of rat blood and cerebral cortex were 6.14±0.38 μg/ml and 1.45±0.09 μg/g, respectively, at 15 min after tetramethylpyrazine administration (10 mg/kg, i.v.).

program. The pharmacokinetic parameters, as derived from the data and calculated by the PCNONLIN program, are shown in Table 4.

Fifteen minutes after administration (10 mg/kg, i.v.), the cerebral cortex concentration and plasma concentration of tetramethylpyrazine were 1.45±0.09 μg/g and 6.14±0.38 μg/ml ($n=5$), respectively. There were no significant differences (Newman Keuls test with the level of significance set at $p<0.05$) in tetramethylpyrazine concentration among the various regions of the brain. The mean peripheral plasma concentration was approximately five-times that of brain tissue.

4. Conclusions

The method was used for simultaneous monitoring of the concentration of tetramethylpyrazine in rat plasma and brain tissue sample. In the pharmacokinetic study, the disposition of tetramethylpyrazine was characterized according to two-compartment open model. No significant differences in tetramethylpyrazine concentration were found between various regions of the rat brain (cerebral cortex, cerebellum, hippocampus, brain stem, striatum and midbrain) 15 min after tetramethylpyrazine administration (10 mg/kg, i.v.).

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Table 4
Pharmacokinetic parameters of tetramethylpyrazine administered to rat (2, 5, or 10 mg/kg, i.v.; $n=5$)[a]

Parameter	Dose (mg/kg)		
	2	5	10
A (μg/ml)	1.21±0.35	3.73±0.55	5.94±0.89
B (μg/ml)	0.70±0.14	2.74±0.37	3.89±0.98
α (1/min)	0.29±0.04	0.11±0.01	0.10±0.03
β (1/min)	0.038±0.005	0.032±0.002	0.026±0.004
$t_{1/2,\alpha}$ (min)	2.6±0.3	6.4±0.7	9.2±2.0
$t_{1/2,\beta}$ (min)	19.7±3.1	21.6±1.1	28.0±3.4
AUC (μg min/ml)	23.1±3.0	118.4±4.3	227.0±18.5
CL (ml/min/kg)	92±11	42±2	45±3

[a] Data are expressed as mean±S.E.M. AUC=Area under the concentration curve, CL=clearance. See text for other abbreviations.

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